

Morphological and productivity comparison between commercial and wild isolates of *Pleurotus eryngii* (D.C.: Fr.) Qué!

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Abstract

Seven commercial isolates of *Pleurotus eryngii* ('142 F', '142 E', 'D +', 'V turbo', 'V 142', 'A12' and 'V160'), identified on molecular basis, were compared to five wild type isolates ('Albino beige chiaro 1094', 'Altamura 1603', 'Albino 1107', 'Muro Lucano 139' and 'Conversano 1250') concerning their morphological, productivity and quality features. Interestingly, two wild type isolates ('Albino 1107' and 'Conversano 1250') resulted more productive than all the commercial fungal isolates analysed. Besides, all wild isolates of *P. eryngii* showed harvest earliness and among them 'Altamura 1603' and 'Conversano 1250' were the best. The highest percentage of large basidioma was observed for the commercial isolates 'V turbo' and 'D+'. Within the wild types, the isolate 'Conversano 1250' stood out. Regarding the pileus cuticle colour, commercial isolates ('V turbo' and 'V 160') showed lower lightness value than wild type isolates ('Albino 1107').

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Introduction

Mushrooms are foods with high nutritional value and health benefits thanks to their low caloric intake, fat content and a good supply of minerals and vitamins along with antitumoral properties (Coli *et al.*, 1988; Jong and Donovich, 1989; Hobbs, 1995; Chang *et al.*, 1996; Manzi *et al.*, 1999; Guzmán, 2000; Muszyńska *et al.*, 2017). For this reasons, many studies were undertaken to recognise edible fungi, investigate the quality features, improve the production techniques, clarify their taxonomy and diseases (Ferri, 1986; Zervakis and Balis, 1996; Sisto *et al.*, 1998; Ferri, 2000; Maddau *et al.*, 2000; De Gioia *et al.*, 2007; Ferri *et al.*, 2007; Camele *et al.*, 2010; Mang and Figliuolo, 2010; Rodriguez Estrada *et al.*, 2010; Kim *et al.*, 2013; Zhang *et al.*, 2014; Zervakis *et al.*, 2014; Venturella *et al.*, 2015; Gorai and Sharma, 2018).

As edible fungi, *Pleurotus eryngii* (D.C.: Fr.) Qué! is undoubtedly one of the most known and cultivated mushroom from the past. It is also very much appreciated throughout all Italy, and in the Southern region it is commonly referred as *cardoncello*. In the last decades, the cultivation of this fungus has assumed an ever-increasing expansion and diffusion worldwide (Ferri, 1986; Zhang *et al.*, 2014; Ryu *et al.*, 2015). Furthermore, for *P. eryngii* various beneficial effects on human health have been demonstrated thanks to the hypocholesterolemic and hypoglycaemic properties and several studies have confirmed their antioxidant, immunomodulatory, antiosteoporotic and antitumor properties (Jong and Donovich, 1989; Grunde-Cimerman, 1999; Guzmán, 2000; Wang and Ng, 2001; Ngai and Ng, 2006; Muszyńska *et al.*, 2017).

Our studies were directed to discover new promising *P. eryngii* isolates with better marketable features compared with the already commercially available ones. For this purpose, seven commercial and five wild *P. eryngii* isolates, identified at species level based on molecular basis, were evaluated for their morphological, productivity and some quality characteristics.

Materials and methods

Fungal isolation, inoculation and culture

The isolates of *P. eryngii* used in this study are shown into Table 1 and Figure 1. All isolates investigated were stored as pure fungal cultures at 4°C on potato dextrose agar (PDA) media into the fungal culture collection of the mycology laboratory at University of Basilicata (UNIBAS), Potenza (Italy). The studied isolates were identified at species level by molecular methods using polymerase chain reaction (PCR) employing the primer pair ITS5/ITS4 (White *et al.*, 1990), which amplify the internal transcribed spacer (ITS) 1 and 2 regions including the 5.8S rRNA gene of the ribosomal RNA. Genomic DNA isolation and PCR

were performed as described by Mang and Figliuolo (2010). The obtained amplicons were directly sequenced by BMR S.r.l. Genomics Padova (Italy). The resulted sequences were analysed using the basic local alignment search tool (BLAST) (Altschul *et al.*, 1990) program and compared to the ITS nucleotide sequences already present in GenBank. The obtained sequences were deposited in the EMBL nucleotide database. Both, commercial and wild types of *P. eryngii* isolates were re-transferred to Petri dishes containing PDA and incubated at 22°C for 24 days. After incubation, the mycelium was suitable for the wooden sticks inoculation. Subsequently, twelve glass cylinders were prepared (one/isolate), containing 10 wooden sticks (well colonised by fungal mycelium) each and placed in an incubator at 22°C in dark. Packs of inoculated standard substrate, were prepared and put into cultivation in the horticulture laboratory of the UNIBAS. A total number of 72 substrate bags (6/isolate, 4.5 kg each) were used for inoculation with the wooden sticks. When the growth of the fungal mycelia became abundant and/or pinheads emerged, portions of the bags were cut-off to create perforations in order to facilitate the fruiting bodies development. Inoculated substrate bags were placed in a greenhouse at 25°C 5±2°C equipped with a black net overshadowing (75%) on the roof. The cultivation of cardoncello took place following the technique described elsewhere by Rana *et al.* (2013). Relative humidity was maintained at ≈80% by spraying tap water. Experimental trials were monitored in order to observe the primordial formation and to evaluate the different growth rate of the *P. eryngii* isolates tested.

The cultivation started on October 10, 2016 (after a 4-day incubation of the substrate bag at 8°C) and ended on November 24, 2016. A total number of twelve experimental trials were carried out for the assay. We have applied a randomised block experimental design with three replicates, and each experimental unit included two substrate bags well colonised by mycelium. Basidioma harvest for all isolates was done in the first basidioma flush since it is generally the most consistent and usually corresponding to about 70-90% of the total production while the other flushes typically lead to less production (≈10-30%). As stated by Ohga and Royse (2004), in general, the mushroom producers only harvest the 1st flush of mushrooms.

Biometric analysis

For each *P. eryngii* isolate, experimental trial and repetition the biometric parameters were evaluated. Also, on a representative sample (5 basidioma/unit) the fresh weight was determined and the dry weight. Drying of the material was done in a ventilated stove at 60°C temperature, until the constant weight was reached. The following biometric parameters were assessed: i) production in the 1st flush (days); ii) average harvest time in days; iii) percentage of large basidioma (with cap diameter ≥3 cm); iv) basidioma medium weight (g); v) basidioma height (cm); vi) stem diameter (cm); vii) stem height (cm); viii) cap diameter (cm); ix) cap consistency (kg cm⁻²) and x) dry matter (%).

Colorimetric analysis

The colour of basidioma pileus cuticle was detected with a Minolta CR-400 electronic colorimeter (Minolta Corp., Osaka, Japan). Colour parameters were expressed as L* describing lightness (L* = 0 for black, L* = 100 for white), a* describing intensity in green-red (a < 0 for green, a > 0 for red), b* describing intensity in blue-yellow (b < 0 for blue, b > 0 for yellow) (Buckley and Giorgianni, 2016). Other colour components such as Chroma (C*) and the Hue angle (H°) were also calculated using specific math

formulas (Castronuovo *et al.*, 2015). Chroma measures colour saturation or intensity and the H° describes the relative amounts of redness and yellowness where 0°/360° is defined for red/magenta, 90° for yellow, 180° for green and 270° for blue colour or purple (Konica Minolta, 2003).

Statistical analysis

All data were statistically analysed by ANOVA and mean values were separated by the Student-Newman-Keuls (SNK) test at P≤0.05 using the SAS software (SAS Institute, Cary, NC, USA, 2015).

Results and conclusions

The PCR reaction performed with ITS5/ITS4 primers produced, in all isolates, a single amplicon of about 600 bp in length permitting direct sequencing of the ITS region. The BLAST analysis of the obtained ITS nucleotide sequences in NCBI GenBank showed a 99%-100% similarity with the ITS sequences of *P. eryngii* already present in GenBank (MH854697; JQ837483 and FJ904728) confirming that all isolates studied belonged to *P. eryngii*. The *P. eryngii* ITS nucleotide sequences from this study were deposited in the EMBL nucleotide database under the accession numbers from LR633968 to LR633979 (Table 1).

The production, obtained for the 12 *P. eryngii* isolates analysed was highly variable, covering a wide range from 120 g ('Albino beige chiaro 1094') till 865.13 g ('Albino 1107') as shown in Table 2. The highest production of commercial isolates was obtained for 'V 160' (758.86 g) and '142 F' (739.69 g) and for the wild type isolates 'in Albino 1107' (865.13 g) and 'Conversano 1250' (863.96 g). Similar values were obtained in previous studies by Rana *et al.* (2013). The harvest earliness was very different among the commercial and wild type cardoncello isolates. The best values were registered for the wild type isolates 'Conversano 1250' (10.0 days) and 'Altamura 1603' (10.0 days) compared to commercial '142 E' isolate (17.32 days). Outcomes on harvest earliness from this study were different from those of previous studies on *P. eryngii* isolates performed by Rana *et al.* (2013) which reported no significant differences regarding the yield earliness

Table 1. Details on *Pleurotus eryngii* isolates used in this study.

Isolate name	Type	GenBank accession number
'142 F'	Commercial	LR633968
'142 E'	-	LR633969
'D +'	-	LR633970
'V turbo'	-	LR633971
'V 142'	-	LR633972
'A 12'	-	LR633973
'V 160'	-	LR633974
'Albino beige chiaro 1094'	Wild	LR633975
'Altamura 1603'	-	LR633976
'Albino 1107'	-	LR633977
'Muro Lucano 139'	-	LR633978
'Conversano 1250'	-	LR633979

among all tested isolates.

The highest percentage of large basidioma was observed for the commercial isolates 'V turbo' (96.29%), 'D+' (95.76%) and '142 E' (93.44%) while the lowest values were seen in the wild type isolates 'Muro Lucano 139' (29.08%) and 'Albino 1107' (31.48%). The average weight of basidioma exceeded 60 g for the commercial isolates '142 F' and 'V 160', while another commercial isolate 'A 12' isolate produced basidioma of smaller size (27 g); in all other commercial isolates the weight of the basidioma ranged from 53 g for 'V turbo' to 41.2 g for 'V 142'. Besides, the wild type isolates 'Altamura 1603' (42.5 g) and 'Conversano 1250' (40.5 g) had the highest average weight of the basidioma while the 'Albino 1107' (17.3 g) wild type had the lowest one (Table 3).

Commercial isolates '142 E' (8.78 cm) and 'D+' (8.74 cm) distinguished themselves by the greater diameter of the stem along with '142 F' (8.52 cm). The smallest values were registered for the wild type isolates 'Albino beige chiaro 1094' and 'Albino 1107' both having a stem diameter of 4.13 cm. The highest height cap values was registered for the 'Muro Lucano 139' wild type isolate (9.79 cm) closely followed by the commercial isolate '142 E' (9.29 cm) while the lowest values for the cap height was registered for the 'A 12' commercial isolate (6.23 cm). The maximum values for the stem height were registered for the wild type *P. eryngii* isolates 'Albino 1107' (5.59 cm) and 'Muro Lucano 139' (5.01 cm) followed by the commercial '142 F' isolate (4.86 cm). On the opposite, the lowest values for the stem height was registered for the 'A 12' (2.87 cm) commercial isolate.

The 'A 12' isolate was characterised by the almost rounded basidioma shape, having reached the largest diameter of the cap (3.12 cm) while 'Albino 1107' isolate showed the smallest diameter of the cap (1.37 cm). The commercial cardoncello isolates 'V 160', 'V turbo' and '142 E' had almost equal values for the cap diameter ranging from 2.68 to 2.79 cm (Table 3).

The trend of the cap consistency among all isolates analysed was very different and varied from a minimum of 1.1 kg cm⁻² for 'D+' commercial isolate up to a maximum of 4.1 kg cm⁻² for the 'Albino beige chiaro 1094' wild type isolate.

Regarding the dry matter content, the highest percentage was registered in the wild type isolates 'Albino beige chiaro 1094' (24.33%) and 'Muro Lucano 139' (22.65%) and the lowest in two wild type isolates 'Altamura 1603' (10.04%) and 'Conversano 1250' (8.97%), compared to all others that, on average, reached 12.7% (Table 3).

The L* value ranged between 87.43 to 45.97, while a-values (red/green) and b-values (yellow/blue) ranged between 1.87-7.24 and 15.2-24.04, respectively according to Wan-Rosli (2013). The L* values of commercial type *P. eryngii* isolates were lower than wild type isolates, thus more dark and attractive from consumers, in particular 'V turbo' (45.97) and 'V 160' (46.21) compared to

Table 2. Productivity and harvest earliness parameters of *P. eryngii* commercial and wild type isolates.

Isolate name	Parameters*	
	Production [1 st flush] (g)	AHT° (days)
'142 F'	739.69 ^{ab}	20.09 ^{bc}
'142 E'	610.48 ^{ac}	17.32 ^c
'D+'	421.75 ^{bc}	22.03 ^b
'V turbo'	589.44 ^{ac}	23.08 ^b
'V 142'	479.17 ^{bc}	21.15 ^{bc}
'A 12'	239.19 ^{de}	27.52 ^a
'V 160'	758.86 ^{ab}	17.66 ^c
'Albino 1107'	865.13 ^a	12.00 ^d
'Altamura 1603'	723.03 ^{ab}	10.00 ^d
'Muro Lucano 139'	186.87 ^e	17.00 ^c
'Conversano1250'	863.9 ^a	10.00 ^d
'Albino beige chiaro 1094'	115.17 ^e	11.00 ^d

*Values with different superscript are significantly different at P≤0.05 according to the SNK test; °AHT (average harvest time, days) = (w₁.d₁) (w_n.d_n) / P. Where: d = time (days) from the culture initiation; w = basidioma weight (marketable production) of each harvest ('flush'); P = production.

Table 3. Morphological and qualitative parameters of *P. eryngii* commercial and wild type isolates.

Isolate name	Large basidioma° (%)	Medium weight (g)	Basidioma Parameters*			Stem Cap Diameter (cm)	Consistency (kg cm ⁻²)	Dry matter (%)
			Height (cm)	Diameter (cm)	Height (cm)			
'142 F'	90.78 ^{ab}	64.89 ^a	8.34 ^{abc}	8.52 ^{ab}	4.86 ^{ac}	2.41 ^{ac}	1.85 ^{bd}	11.91 ^{bc}
'142 E'	93.44 ^a	47.89 ^{bc}	9.29 ^{ab}	8.78 ^a	4.73 ^{ac}	2.68 ^{ab}	1.61 ^{bd}	12.38 ^{bc}
'D +'	95.76 ^a	41.60 ^{cd}	7.67 ^{ac}	8.74 ^a	3.46 ^{de}	2.00 ^{bc}	1.11 ^d	10.06 ^{cd}
'V turbo'	96.29 ^a	52.98 ^{ac}	7.68 ^{ac}	7.41 ^{ac}	3.79 ^{ce}	2.70 ^{ab}	2.16 ^{bc}	12.25 ^{bc}
'V 142'	86.09 ^{ab}	41.23 ^{cd}	6.86 ^{bc}	7.58 ^{ac}	2.99 ^e	2.10 ^{ac}	1.58 ^{bd}	13.04 ^b
'A 12'	86.78 ^{ab}	27.19 ^{de}	6.23 ^c	6.54 ^{cd}	2.87 ^e	3.12 ^a	1.29 ^{cd}	13.44 ^b
'V 160'	78.76 ^{ab}	60.23 ^{ab}	8.11 ^{ac}	7.06 ^{bc}	4.31 ^{bd}	2.79 ^{ab}	2.38 ^b	13.08 ^b
'Albino 1107'	31.48 ^d	17.34 ^e	7.84 ^{ac}	4.13 ^e	5.59 ^a	1.37 ^c	1.89 ^{bd}	13.75 ^b
'Altamura 1603'	51.06 ^c	42.52 ^{cd}	6.62 ^{bc}	7.00 ^{bc}	3.43 ^{de}	2.00 ^{bc}	1.70 ^{bd}	10.04 ^{cd}
'Muro Lucano 139'	29.08 ^d	29.63 ^{de}	9.79 ^a	5.20 ^{de}	5.01 ^{ab}	2.42 ^{ac}	2.42 ^b	22.65 ^a
'Conversano 1250'	72.60 ^b	40.54 ^{cd}	7.20 ^{ac}	7.15 ^{ac}	3.78 ^{ce}	1.85 ^{bc}	1.39 ^{cd}	8.97 ^d
'Albino beige chiaro 1094'	46.74 ^c	20.22 ^e	6.55 ^{bc}	4.13 ^e	4.28 ^{bd}	1.79 ^{bc}	4.12 ^a	24.33 ^a

*Values with different superscript are significantly different at P≤0.05 according to the SNK test; °large basidioma = basidioma with diameter ≥3 cm.*Values with different superscript are significantly different at P≤0.05 according to the SNK test; °large basidioma = basidioma with diameter ≥3 cm.

'Albino 1107' (87.43). The Hue angle range was between 0° the 90° region which suggests a red-yellow colour (Table 4).

This study showed that great differences were recorded among all *P. eryngii* tested isolates regarding parameters analysed similarly to what stated previously by other authors (De Gioia *et al.*, 2007;

Rana *et al.*, 2013). Since important features as productivity and harvest earliness were very good in some of the wild type *P. eryngii* isolates *e.g.* 'Conversano 1250' and 'Albino 1107' certainly, more investigations on this particular isolates should be planned.

Further investigations are needed to verify the importance of

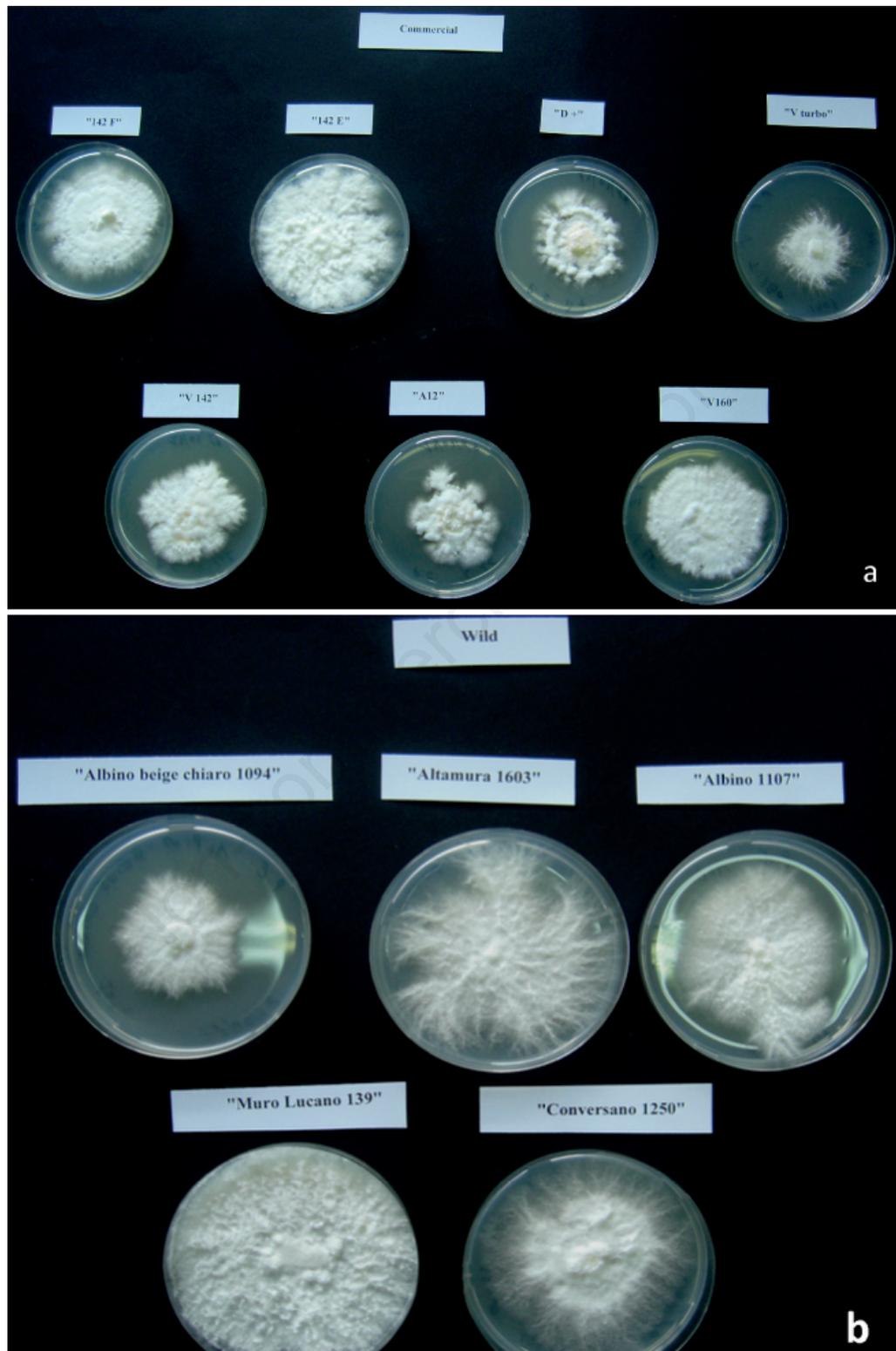


Figure 1. Aspect of the colonies (in different age) of *P. eryngii* isolates commercial (A) and wild type (B) investigated.

Table 4. *Pileus colorimetric characterisation of commercial and wild type P. eryngii isolates.*

Isolates	Colorimetric parameters ^o					
	L*	a*	b*	a*/b*	C*	H
'142 F'	52.26 ^{df}	6.36 ^b	17.14 ^{ce}	0.37 ^{ab}	18.28 ^{ce}	69.64 ^{ef}
'142 E'	50.68 ^{ef}	7.24 ^a	19.35 ^{bc}	0.37 ^{ab}	20.67 ^{bc}	69.44 ^{ef}
'D+'	59.75 ^{cd}	5.85 ^b	18.11 ^{bd}	0.32 ^{bc}	19.04 ^{be}	72.04 ^{de}
'V turbo'	45.97 ^f	5.95 ^b	15.25 ^e	0.39 ^a	16.37 ^e	68.63 ^f
'V 142'	55.36 ^{ce}	6.12 ^b	17.46 ^{ce}	0.35 ^{ac}	18.49 ^{ce}	70.69 ^{df}
'A 12'	54.33 ^{ce}	6.63 ^{ab}	18.62 ^{bd}	0.35 ^{ac}	19.77 ^{bd}	70.42 ^{df}
'V 160'	46.21 ^f	6.35 ^b	16.11 ^{de}	0.39 ^a	17.31 ^{de}	68.50 ^f
'Albino 1107'	87.43 ^a	1.87 ^e	24.04 ^a	0.08 ^f	24.13 ^a	85.63 ^a
'Altamura 1603'	57.42 ^{ce}	6.33 ^b	20.66 ^b	0.31 ^c	21.62 ^b	72.97 ^d
'Muro Lucano 139'	61.09 ^c	4.93 ^c	19.22 ^{bc}	0.26 ^d	19.84 ^{bd}	75.63 ^c
'Conversano 1250'	62.04 ^c	6.02 ^b	19.54 ^{bc}	0.31 ^c	20.45 ^{bc}	72.91 ^d
'Albino beige chiaro 1094'	72.40 ^b	2.98 ^d	19.33 ^{bc}	0.15 ^e	19.56 ^{bd}	81.26 ^b

^oValues with different superscript are significantly different at $P \leq 0.05$ according to the SNK test. Colour indexes: L* = luminosity index; a* = red-green component index; b* = yellow-blue component index; C* = saturation index (chroma); H = colour tone 'Hue'.

the *P. eryngii* colour in consumer preferences. In fact, only few studies (Martínez-Soto *et al.*, 2001; Nour *et al.*, 2011) were found where colour in dried mushrooms had been linked to consumer preference but this hypothesis was not supported by experimental evidence.

Further investigations are needed to better generalise the results from this study. It will be necessary to take into account a greater number of isolates, especially wild type ones, and to test them in a larger variety of growth conditions. Another possible direction is to explore the biodiversity of *P. eryngii*, discovering new fungal isolates.

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